CCXXIV. THE METABOLISM OF GALACTOSE.

II. THE SYNTHESIS OF LACTOSE BY SLICES OF ACTIVE MAMMARY GLAND *IN VITRO*.

By GORDON ALLISON GRANT¹.

From the Department of Biochemistry, Lister Institute, London.

(Received June 27th, 1935.)

ONE of the most interesting natural transformations in carbohydrate metabolism is that occurring in the active mammary gland during the synthesis of the galactose-containing disaccharide, lactose. Rohmann [1919] was able to obtain some evidence of the synthesis of lactose by the action of an extract of the mammary gland upon added sucrose. This investigator, on what now seems inconclusive evidence, suggested a stereokinase system, present in the blood as well as the gland and capable of causing the interconversions: glucose $\rightarrow$ fructose $\rightarrow$ galactose. More recently, Michlin and Lewitow [1934], using the minced mammary gland of lactating cows, have obtained an enzymic synthesis from added glucose and galactose of a disaccharide, which is hydrolysed by emulsin and is presumably lactose. Bert [1884] and later Porchet [1909] claimed that in animals which had previously had the mammary gland removed and subsequently became pregnant, hyperglycaemia developed and the sugar appearing in the urine was not lactose but glucose. Widmark and Carlens [1925] reported hypoglycaemia in lactating cows during milking, the degree roughly corresponding to the amount of milk produced. Paton and Cathcart [1911] obtained evidence to indicate that the glucose of the blood is used in the mammary gland during the formation of lactose. Harding and Downs [1929] were unable to demonstrate a lowered blood sugar in freely lactating women while on a hospital diet. They expressed the view that the amount of glucose circulating in the blood is controlled by the internal secretions rather than conditioned entirely by the lactose output of the active mammary gland. Nitzescu [1925] has been able to show that in fasting there is a drop in the lactose concentration of the milk produced by animals in full lactation. Upon the intravenous injection of glucose, galactose, fructose or maltose, the normal level of the milk lactose is restored. Injection of sucrose or lactose was without effect—these sugars are not utilised since there is no effective concentration of sucrase or lactase in the blood—and the disaccharides administered are, for the most part, excreted into the urine, owing to the low renal threshold for these sugars. The results of Nitzescu are explicable equally well by the assumption of a direct synthesis of lactose from the sugars administered by the active mammary gland, or by the previous conversion of these sugars into glucose, by way of glycogen formation in the liver.

Thus the available physiological evidence suggests that the freely lactating gland is apparently capable of withdrawing glucose from the blood and converting it into the milk sugar, lactose. The gland is automatically supplied with a surplus of blood sugar to meet the demands of milk formation, the stimulus emanating from some other source than the mammary glands themselves.

¹ Beit Memorial Research Fellow.

It is considered that the development of the mammary glands occurs under the influence of oestrin and progesterin, and that the hypophyseal prolactin then causes the actual secretion [cf. Turner and Gardner, 1931; Turner and Frank, 1932; Gardner and Turner, 1933; Riddle *et al.*, 1933; Allen *et al.*, 1935; Lyons *et al.*, 1933].

Nelson [1934] and Nelson and Smelser [1933] suggest that a sudden fall in the amount of the ovarian hormone may act as a stimulus to the hypophysis for prolactin secretion. Selye *et al.* [1934] claim that the act of suckling reflexly maintains an adequate prolactin secretion; if this is interrupted by hypophysectomy lactation ceases. Grauer and Robinson [1932] observed milk secretion in transplanted mammary tumours, which is evidence that the nervous mechanism is not essential to milk production.

In the present investigation lactose synthesis has been demonstrated and quantitatively investigated with tissue slices of the active mammary gland, maintained *in vitro* under conditions as nearly as possible physiological, using as substrates the hexoses, glucose, fructose, mannose and galactose. The mechanism seems to a large extent specific, for under conditions of almost complete conversion of glucose into lactose only a small amount of synthesis occurred from fructose, mannose or galactose.

EXPERIMENTAL.

Mammary glands of freely lactating guinea-pigs were removed at periods from 2 to 5 days after the birth of the young. The animals were kept with the young until the time of the experiment, since this ensures an active stimulus to milk formation, coupled with the minimum amount of residual milk in the gland. A single gland was used for each of the four series of experiments, in order to obtain as nearly as possible comparable results with the different sugars. The tissue slices (approximately 0.5 mm. thick) were washed once in a physiological salt solution (10 : 3 : 0 [Robison and Rosenheim, 1934]) kept at 37°, and saturated with a mixture of O₂, 95 %; CO₂, 5 %. After quickly removing the excess solution by means of filter-paper, the slices were transferred to 25 ml. flasks, in some of which were 2 ml. of the above basal solution, saturated with the O₂-CO₂ mixture, whilst others contained in addition 0.4 % (approximately) of the particular sugar under investigation. The flasks, tightly stoppered, were kept in a thermostat at 37°, with constant shaking, for a period of 6 hours. The wet weight of the tissues was obtained by weighing the flasks before and after the addition of the tissue slices and again at the end of the experiment. The dry weight was calculated from the H₂O determination (3 hours at 110°) carried out on aliquot slices of the gland in each of the four series. At the end of the experiment the supernatant fluid was transferred to a 20 ml. flask and the tissue slices were removed and ground with sand in a mortar and quantitatively transferred to the same flask; 1 ml. of 10 % ZnSO₄.7H₂O solution was added, followed by 1 ml. of 0.5N NaOH, added slowly with shaking. The contents of the flask were diluted to 20 ml. and filtered. The sugar estimations were carried out on these protein-free filtrates; in the fructose and galactose experiments a 1 in 2 dilution of these filtrates was used.

Estimation of the hexoses and lactose.

The fractionation of the reducing substances of the deproteinised filtrates into "fermentable sugar" and galactose was carried out by the general method of Harding and Grant [1933]. In the present investigation the method has been extended to include the quantitative estimation of lactose. All sugar reductions

were carried out with the Harding and Downs [1933] modification of the Shaffer-Hartmann Cu reagent using the requisite factor for the sugar concerned.

1. *Fermentable sugar* (glucose, fructose or mannose). 0.25 g. (wet weight) of washed baker's yeast was added to 10 ml. of the mammary gland filtrate and incubated with stirring for 30 minutes at 37°. Sugar estimations were carried out on 1 ml. portions of the clear centrifugate. This treatment removes glucose, fructose and mannose. The amount is estimated by difference in reducing power.

2. *Galactose*. 0.25 g. (wet weight) of "galac" yeast was added to the residual centrifugate and incubated for 30 minutes at 37° as in 1. The "galac" yeast used was a strain of English mild ale top yeast which had been adapted to ferment galactose at 80–100 % the rate for glucose [Grant, 1935]. This treatment removes galactose, and the amount is determined by further decrease in reducing power. In the filtrates from the galactose experiments, 0.5 g. of the yeast was employed.

3. *Lactose*. It has been found possible to remove lactose from its dilute solutions, in a similar manner, by the use of *S. fragilis*, a lactose-fermenting yeast. The yeast was grown on a 4 % fructose-4 % lactose mixture in a yeast extract, phosphate medium. 0.5 g. (wet weight) of the washed *S. fragilis* was added to the residual centrifugate after removal of the galactose and incubated as before at 37° for 30 minutes. By this treatment 90–100 % of added lactose is removed in concentrations up to 30–40 mg. per 100 ml.

Table I. *The synthesis of lactose from different hexoses by slices of active mammary gland in vitro. (6 hours at 37°.)*

No.	Sugar added	Time hours	Amount of tissue (dry wt.) mg.	Hexose added mg.	Hexose remaining at end of experiment mg.	Lactose found mg.	Lactose found as mg. per 100 mg. dry tissue
1	Control	0	56	—	—	1.54	2.75
	"	"	106	—	—	1.50	1.41
	"	6	32	—	—	0.23	0.72
	"	"	93	—	—	0.38	0.41
	Glucose	"	56	7.05	0.81	2.91	5.20
	"	"	40	7.05	0.80	3.24	8.10
	"	"	47	7.05	0.29	3.31	7.04
	"	"	51	7.05	0.43	3.21	6.29
	"	"	"	"	"	"	"
2	Control	0	107	—	—	0.03	0.03
	"	6	56	—	—	0	0
	"	"	175	—	—	0.07	0.04
	Glucose	"	65	7.88	2.31	1.24	1.90
	Mannose	"	50	8.37	4.02	0.40	0.80
	Galactose	"	67	7.84	5.46	0.20	0.30
	Fructose	"	79	8.12	7.61	0.20	0.25
3	Control	0	117	—	—	1.49	1.36
	"	"	97	—	—	1.49	1.53
	"	6	90	—	—	0.67	0.74
	"	"	58	—	—	0.53	0.91
	Glucose	"	92	7.88	0.07	4.98	5.41
	Mannose	"	91	8.37	2.39	1.49	1.64
	Galactose	"	65	7.84	6.00	1.14	1.75
	Fructose	"	48	8.12	6.54	0.60	1.25
4	Control	0	126	—	—	1.34	1.63
	"	6	83	—	—	0.50	0.62
	"	"	71	—	—	0.67	0.94
	Glucose	"	84	8.72	1.28	4.68	5.57
	"	"	60	8.72	1.64	4.31	7.18
	Mannose	"	81	7.82	4.09	1.03	1.27
	Galactose	"	52	7.94	5.60	0.28	0.54
	Fructose	"	87	8.58	6.85	1.00	1.15

The different yeasts can be kept at 4°, in the form of their 25 % wet weight suspension for 3 to 4 weeks and still retain most of their removal activity, provided they are washed at intervals of a few days. Their removal power is tested before the yeasts are used.

The results obtained in the present investigation are summarised in Table I. The amount of hexose added is given in each case, and also that present at the end of the experimental period. The lactose present in the tissues at the beginning of the experiment and at the conclusion is shown, and can be compared with the amount of this sugar obtained when the tissue slices were kept immersed in the solutions of the different hexoses, glucose, fructose, mannose and galactose.

DISCUSSION.

The results obtained show clearly that the active mammary gland possesses the mechanism required for the rapid synthesis of the galactose-containing disaccharide, lactose, from added glucose. There was but little evidence of such synthesis from the other hexoses investigated, fructose, mannose and galactose. Mannose appears to be more readily utilised, for the above purpose, than either galactose or fructose, though the difference is slight. It may be that mannose, fructose, and galactose require first to be converted into glucose, perhaps by the path of glycogen formation, before they can act as substrates for the synthesis of lactose. Lactose disappears from the mammary gland slices during the time of the experiment, and added lactose also undergoes a slow metabolism. For this reason the amount of lactose found does not represent the total synthesis occurring. However, from the amount of the added hexose remaining at the end of the experimental period, it is possible to obtain some idea of the amount which has been metabolised in excess of that finally present as lactose. This is considerable in the case of glucose but much less with the other three sugars. The small amount of fructose utilised by the active gland preparations, either in metabolism or for the synthesis of lactose, is especially noteworthy.

Svanberg [1930] found that an enzymic preparation of the parenchyma of the udder of heavy-milking cows could produce lactic acid from glucose, galactose, a mixture of the two and from lactose. Barrenscheen and Alders [1932] confirmed these findings and claimed that the capacity to produce lactic acid from galactose and lactose was a new function of the active mammary gland. They also suggested that hexosephosphates might constitute the intermediate material for the glucose-galactose conversion in the gland. Börst [1932] believes that the mammary gland stores organic phosphates in the periods between active lactation. In mammary tissues, and in many others, he found a phosphatase which could eliminate P from adenylic acid. This investigator considers that adenylic acid is important in the synthesis of lactose. Brenner [1932] found that while "lactacidogen" breakdown is complete in 2 hours, it takes 6 hours for the increase of inorganic P to cease during the autolysis of mammary gland tissue. Folley [1935] suggests that the phosphatase of the active mammary gland is probably identical with that of the kidney. He showed that it is able to catalyse the synthesis of sodium glycerophosphate.

Unpublished experiments of the present author have shown that the phosphatase of the lactating gland of the rabbit and the guinea-pig hydrolyses at approximately equal rates the following naturally-occurring hexosephosphates, 1:6-diphosphofructofuranose, glucose-6-phosphate, fructose-6-phosphate, trehalosemonophosphate and the synthetic ester, galactose-6-phosphate. It has been difficult to demonstrate synthesis of organic phosphates from added glucose and inorganic phosphate either by a "brei" or by tissue slices of the secreting

gland. A slight synthesis was observed in one instance in the presence of added hexokinase from yeast.

It is not yet possible to decide whether the phosphorylation taking place is connected only with the glycolysis of the active tissue, or whether it is also necessary in the series of changes taking place in the conversion of the glucose into lactose. It is of course quite possible that a new enzyme is formed by the lactating tissue for the conversion of the glucose to galactose, prior to the synthesis of the lactose.

In the experiments with galactose, there was no evidence of the reverse change of galactose $\rightarrow$ glucose. Nor was there any evidence of the formation of noticeable amounts of free galactose from the hexoses investigated; the small quantities of this sugar found in some cases appeared equally in the control experiments. From this preliminary investigation therefore no evidence has emerged of the synthesis of galactose in the free state, but only of this sugar combined through its first C atom to the 4th atom of a molecule of glucose forming lactose.

SUMMARY.

The extent of the synthesis of the galactose-containing disaccharide, lactose, by the active mammary gland, when tissue slices of the lactating gland are kept immersed in physiological salt solutions containing different hexoses, has been determined. Of the hexoses used as substrates, glucose is readily converted into lactose, whilst there is but little evidence of synthesis from fructose, mannose and galactose. The lactose was estimated by the use of *S. fragilis*. Evidence was obtained of the presence of a hexosephosphatase in the active gland. A slight synthesis of organic phosphates from added glucose and phosphate was obtained in one experiment, when hexokinase from yeast was present.

REFERENCES.

- Allen, Gardner and Diddle (1935). *Endocrinology*, **19**, 305.
 Barrenscheen and Alders (1932). *Biochem. Z.* **252**, 97.
 Bert (1884). *Compt. Rend. Acad. Sci.* **98**, 775.
 Börst (1932). *Z. physiol. Chem.* **212**, 126.
 Brenner (1932). *Z. physiol. Chem.* **212**, 135.
 Folley (1935). *J. Soc. Chem. Ind.* **54**, 86.
 Gardner and Turner (1933). *Missouri Agric. Exp. Sta. Bull.* 196.
 Grant (1935). *Biochem. J.* **29**, 1661.
 Grauer and Robinson (1932). *Amer. J. Cancer*, **16**, 191.
 Harding and Downs (1929). *J. Biol. Chem.* **84**, 335.
 ——— (1933). *J. Biol. Chem.* **101**, 487.
 ——— and Grant (1933). *J. Biol. Chem.* **99**, 629.
 Lyons, Chaikoff and Reichert (1933). *Proc. Soc. Exp. Biol. Med.* **31**, 303.
 Michlin and Lewitow (1934). *Biochem. Z.* **271**, 448.
 Nelson (1934). *Endocrinology*, **18**, 33.
 ——— and Smelser (1933). *Amer. J. Physiol.* **103**, 374.
 Nitzescu (1925). *Compt. Rend. Soc. Biol.* **93**, 1319.
 Paton and Cathcart (1911). *J. Physiol.* **42**, 179.
 Porchet (1909). *Arch. int. Physiol.* **8**, 356.
 Riddle, Bates and Dykshorn (1933). *Amer. J. Physiol.* **105**, 191.
 Robison and Rosenheim (1934). *Biochem. J.* **28**, 684.
 Rohmann (1919). *Biochem. Z.* **93**, 237.
 Selye, Collip and Thompson (1934). *Endocrinology*, **18**, 237.
 Svanberg (1930). *Z. physiol. Chem.* **188**, 207.
 Turner and Frank (1932). *Missouri Agric. Exp. Sta. Bull.* 174.
 ——— and Gardner (1931). *Missouri Agric. Exp. Sta. Bull.* 158.
 Widmark and Carlens (1925). *Biochem. Z.* **158**, 3.